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Autograft Contamination During Preparation for Anterior Cruciate Ligament Reconstruction

By Michael E. Hantes, MD, Georgios K. Basdekis, MD, Sokratis E. Varitimidis, MD, Dimitrios Giotikas, MD, Efthimia Petinaki, MD, and Konstantinos N. Malizos, MD

Investigation performed at the Departments of Orthopaedics and Microbiology, University Hospital of Larissa, Medical School, University of Thessalia, Larissa, Greece

Background: The autograft preparation process for anterior cruciate ligament reconstruction has a potential for graft contamination. The purpose of this study was to evaluate the possibility of contamination of the bone-patellar tendon-bone and hamstring tendon autograft during preparation for anterior cruciate ligament reconstruction.

Methods: A primary isolated reconstruction of the anterior cruciate ligament with use of bone-patellar tendon-bone autograft (thirty patients) and hamstring tendon autograft (thirty patients) was performed in a prospective, consecutive series of patients. Three tissue samples were obtained for culture from each graft at different time-intervals during the graft preparation. In addition, the erythrocyte sedimentation rate and the C-reactive protein level were evaluated preoperatively and on the third, seventh, and twentieth postoperative days, and the clinical course of all patients was monitored.

Results: The time needed for graft preparation was significantly longer for hamstring autografts (nineteen minutes) than for bone-patellar tendon-bone autografts (ten minutes) ($p = 0.032$). In the hamstring group, cultures of graft tissue from four patients (13%) were positive for bacteria. In the bone-patellar tendon-bone group, cultures of graft tissue from three patients (10%) were positive for bacteria; the difference between groups was not significant ($p = 0.923$). No patient had development of a postoperative infection. There were no differences between patients with a contaminated graft and those with an uncontaminated graft with regard to postoperative changes in the erythrocyte sedimentation rate or the C-reactive protein level at all time-intervals.

Conclusions: A high rate (12%) of autograft contamination can be expected during autograft preparation for anterior cruciate ligament reconstruction. The contamination rate is almost equal for both bone-patellar tendon-bone and hamstring tendon autografts. We could not identify an association between contaminated grafts implanted in the knee and postoperative inflammatory markers such as the erythrocyte sedimentation rate and the C-reactive protein level.

Level of Evidence: Therapeutic Level II. See Instructions to Authors for a complete description of levels of evidence.

Arthroscopic reconstruction of the anterior cruciate ligament is a common procedure among orthopaedic surgeons. The bone-patellar tendon-bone autograft and the hamstring tendon autograft are the most popular grafts used for anterior cruciate ligament reconstruction¹. Both grafts require preparation after harvesting (usually carried out at a side table by an assistant). Graft contamination is possible, but, as far as we know, there are no data regarding the rates of bacterial inoculation during graft preparation for anterior cruciate ligament reconstruction. Surgical masks, gloves, and instruments can be a source of contamination as has been shown

by Davis et al.². A contaminated graft implanted in the knee could be a risk factor for septic arthritis, which is a rare but devastating complication following anterior cruciate ligament reconstruction^{3,4}. Since infection adds substantial morbidity, additional research is required to identify risk factors associated with this complication.

Postoperatively, clinical infection can be signaled by changes in routine laboratory screening tests, such as the erythrocyte sedimentation rate and the C-reactive protein level⁵⁻⁷. Both the erythrocyte sedimentation rate and C-reactive protein level are markedly elevated in patients with septic arthritis after

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TABLE I Bacteria Identified in Patients with a Contaminated Graft

Case	Type of Graft	Organism	Sample 1	Sample 2	Sample 3
1	Bone-patellar tendon-bone	<i>Staphylococcus epidermidis</i>	–	+	+
2	Bone-patellar tendon-bone	Streptococcus	+	+	+
3	Bone-patellar tendon-bone	<i>Staphylococcus epidermidis</i>	–	–	+
4	Hamstrings	<i>Staphylococcus epidermidis</i>	+	+	+
5	Hamstrings	<i>Staphylococcus aureus</i>	–	+	+
6	Hamstrings	Acinetobacter	–	+	+
7	Hamstrings	<i>Staphylococcus aureus</i>	–	–	+

anterior cruciate ligament reconstruction^{4,8-10}. However, the time needed for the values to return to normal levels in patients with an implanted contaminated graft is not known.

The primary goals of this prospective study were to evaluate the possibility of bone-patellar tendon-bone or hamstring tendon autograft contamination during preparation for anterior cruciate ligament reconstruction and to identify any association between graft contamination and the development of a clinical infection. Our hypothesis was that the rate of contamination would be higher for hamstring grafts because of the longer graft-preparation time. A secondary aim was to investigate whether there was any association between contaminated grafts and inflammatory blood markers, such as erythrocyte sedimentation rate and C-reactive protein level.

Materials and Methods

The inclusion criteria for the study were (1) a scheduled isolated primary arthroscopic reconstruction of the anterior cruciate ligament and (2) no previous knee surgery. Patients with a collagen disease, immunodeficiency, diabetes, or other metabolic disorders were excluded from the study. Finally, patients with any type of clinical infection (such as influenza) within the two months before surgery were excluded from the study. Sixty consecutive patients who were seen between March 2004 and February 2005 met the inclusion criteria and were eligible for the study. Institutional review board approval was obtained, and all patients signed our informed consent form.

The patients were randomly assigned (with the use of sealed envelopes) to two groups: the hamstring group, which consisted of thirty patients (twenty-seven male and three female patients) and the bone-patellar tendon-bone group, which also consisted of thirty patients (twenty-eight male and two female patients).

Arthroscopic reconstruction of the anterior cruciate ligament with use of a bone-patellar tendon-bone or hamstring autograft was performed in all patients by the senior author (M.E.H.). The graft was harvested at the beginning of the operation. Graft preparation was carried out at a side table by an assistant. Grafts were held in wet sponges without antibiotic solutions after their preparation. All patients received antibiotic prophylaxis with 1 g of amikacin and 2 g of ceforanide administered one hour before surgery and 2 g of ceforanide

administered postoperatively every twelve hours for twenty-four hours. The scheme of antibiotic prophylaxis was in accordance with the sensitivity of bacteria in our region, which had been demonstrated in previous studies^{11,12}. Patients were discharged from the hospital on the third postoperative day. Usually our patients are discharged from the hospital the day following an anterior cruciate ligament reconstruction. However, we extended the hospital stay only for the purposes of this study because most of the patients came from remote areas, which would have made it difficult to obtain the C-reactive protein level and erythrocyte sedimentation rate tests on the third postoperative day. Normal values in our laboratory were 0 to 20 mm/hr for the erythrocyte sedimentation rate and <1 mg/L for the C-reactive protein level. The rehabilitation protocol was the same for all patients and included no limitation of knee motion and weight-bearing as tolerated. Patients were followed clinically for one, three, six, twelve, twenty-six, and fifty-two weeks postoperatively for the purpose of this study.

Evaluation Protocol

In order to evaluate graft contamination in both groups, three graft tissue samples were obtained for culture from each graft at different time-intervals. The first specimen was obtained at the initiation of the graft-preparation process; the second specimen, at the completion of preparation; and the third specimen, just before graft implantation. All samples were collected in sterile containers and were immediately transferred to the microbiology laboratory for inoculation in blood agar, chocolate blood agar, and nutrient broth for four days. The blood agar and chocolate blood agar were incubated at 37°C in the presence of 6% CO₂. The time needed for graft preparation, as well as the time-interval between harvesting and implantation, was recorded in every case. The erythrocyte sedimentation rate and the C-reactive protein level were evaluated preoperatively and on the third, seventh, and twentieth postoperative days.

Statistical Analysis

The Student t test was performed in order to identify differences in autograft preparation and implantation time between the two graft types. Comparisons of the groups with regard to

TABLE II C-Reactive Protein Levels Preoperatively Through Day Twenty Postoperatively

	Mean C-Reactive Protein Level (mg/L) (range)			
	Preop.	Day 3	Day 7	Day 20
Patients with an uncontaminated graft (n = 53)	0.4 (0-1)	13.28 (7-21)	6.4 (2-8)	0.65 (0.1-1.2)
Patients with a contaminated graft (n = 7)	0.5 (0-1)	13.74 (8-22)	5.9 (2-8)	0.61 (0.1-1.3)
P value	0.652	0.910	0.557	0.604

the erythrocyte sedimentation rate and the C-reactive protein levels preoperatively and on the third, seventh, and twentieth day after surgery were done, with the use of Mann-Whitney U tests. The Fisher exact test was used for comparison of the proportions of contaminated autografts in the two groups. The level of significance was set at $p < 0.05$.

Results

The mean age of the patients was 27.3 years (range, eighteen to thirty-seven years) in the hamstring autograft group and 26.1 years (range, seventeen to thirty-eight years) in the bone-patellar tendon-bone group. No patient had a clinical infection (septic knee arthritis, local wound problems, or cellulitis) develop postoperatively during the one-year observation time. However, a total of seven (12%) of the sixty patients had a positive culture for bacteria. None of these patients received any additional treatment since there were no clinical signs of infection. In the hamstring group, cultures of samples from four patients (13%) were positive for bacteria. The bacteria that were identified are listed in Table I. *Staphylococcus (epidermidis or aureus)* was isolated in the majority of the patients with positive cultures. In the bone-patellar tendon-bone group, cultures of graft tissue from three patients (10%) were positive for bacteria. This difference between groups was not significant ($p = 0.923$). However, post hoc power analysis revealed that the study had a probability of only 27% to find a difference between the groups. This means that the study was not sufficiently powered to show a difference that potentially could be present.

In the hamstring group, cultures were positive in all three samples from one patient, in the second and third samples from two patients, and in only the third sample from one patient. The mean graft-preparation time was nineteen minutes (range, sixteen to twenty-one minutes), and the mean time-interval between graft harvesting and implantation was

thirty-eight minutes (range, twenty-eight to forty-three minutes). In the bone-patellar tendon-bone group, cultures were positive in all three samples from one patient, in the second and third sample from one patient, and in the third sample from one patient (Table I). The mean graft-preparation time was ten minutes (range, nine to fourteen minutes), and the mean time-interval between graft-harvesting and implantation was thirty-five minutes (range, twenty-six to forty minutes). The time for graft preparation was significantly less for the bone-patellar tendon-bone group ($p = 0.032$), but there was no difference between the groups with respect to the time from graft harvest to implantation ($p = 0.134$). A post hoc power analysis revealed that the study had at least 80% power to detect differences for graft preparation and for graft implantation.

One patient with negative cultures was readmitted on the ninth postoperative day because of a knee effusion, knee tenderness, and fever. Joint aspiration revealed a hematoma of the knee joint, and cultures were negative. No further treatment was given to the patient, and the clinical signs resolved without further complication.

Monitoring of the C-reactive protein levels revealed that its value increases immediately after surgery (on the third postoperative day) and then gradually neared preoperative values in all patients. There were no differences in C-reactive protein levels between patients with contaminated grafts and those with uncontaminated grafts at all three stages of follow-up (third, seventh, and twentieth postoperative days) (Table II). The erythrocyte sedimentation rate for all patients increased during the first week after surgery and then decreased slightly but remained elevated. Again, there were no differences between patients with contaminated grafts and those with uncontaminated grafts with respect to the erythrocyte sedimentation rate at the three postoperative stages of observation (Table III).

TABLE III Erythrocyte Sedimentation Levels Preoperatively Through Day Twenty Postoperatively

	Mean Erythrocyte Sedimentation Rate (mm/hr) (range)			
	Preop.	Day 3	Day 7	Day 20
Patients with an uncontaminated graft (n = 53)	11.4 (5-18)	33.8 (19-50)	51.6 (20-64)	29.7 (17-42)
Patients with a contaminated graft (n = 7)	12.8 (6-17)	31.3 (17-47)	48.7 (16-60)	31.4 (15-45)
P value	0.341	0.402	0.946	0.857

Discussion

All operations have the potential for contamination, and Lister¹³ recognized the relationship between contamination and subsequent infection. The prevalence of septic arthritis after anterior cruciate ligament reconstruction has been reported to range from 0.1% to 0.9%^{4,9,10}. However, septic arthritis is a devastating complication because the cost of caring for patients with a postoperative infection is very high, and the result in most of the patients is inferior because of knee stiffness and cartilage damage^{4,9,14}. A possible source of septic arthritis after anterior cruciate ligament reconstruction is a contaminated graft⁹; however, to our knowledge, the rate of contamination for autografts has not been previously assessed.

In this study, we investigated the potential for contamination of the autograft during preparation and found that the rate of contamination of an autograft prior to implantation into the knee is 12%. Davis et al.² found that, during hip and knee arthroplasties, contamination of the skin blades and gloves was 9.4% and 28.7%, respectively. We did not find any significant differences between the bone-patellar tendon-bone grafts and the hamstring grafts with regard to the rate of contamination (10% and 13%, respectively), although the time needed for graft preparation was significantly longer for the hamstring grafts. Therefore, our initial hypothesis that the contamination rate would be higher for hamstring autografts was not confirmed. However, our study was inadequately powered to show a difference. Certainly, preparation of hamstring tendon grafts requires more excessive and prolonged manipulation in comparison with bone-patellar tendon-bone grafts because of the need for muscle and fat tissue removal and tendon-suturing. The time from graft harvest to implantation, which according to our results was the same for both grafts, is probably a more important parameter for tissue contamination. It is worth mentioning that, unlike other orthopaedic procedures in which the operative field is covered with sterile adhesive drapes, during arthroscopic knee surgery, the skin, after initial aseptic preparation, remains uncovered throughout the operation and its residual flora are exposed to the surgeon's gloves and surgical instruments and through them to the graft. This might be a source of inoculation of the bacteria onto the graft.

Several experimental studies have investigated the rate of contamination of patellar tendon autografts or allografts dropped onto the operating-room floor¹⁵⁻¹⁸. According to those studies, the rate of contamination under such circumstances was approximately 60%. The main purpose of those studies was to establish guidelines for graft-cleansing in the unusual situation of an accidental contamination. According to Izquierdo et al.¹⁹, 88% of forty-nine surgeons surveyed experienced this problem once in their career. Therefore, this is a very rare event and certainly does not reflect the daily practice of anterior cruciate ligament reconstruction. In contrast, our study provides information about the rate and type of bacterial contamination that can occur during autograft preparation. To our knowledge, this is the first report to evaluate the rate of autograft contamination during the preparation of the graft

and not because of accidental contamination. Díaz-de-Rada et al.²⁰ reported a similar rate of contamination (13%) but only for bone-patellar tendon-bone allografts; however, their findings are not comparable with ours because contamination while harvesting or storing the allografts could have gone undetected^{21,22}.

We did not find any association between graft contamination and postoperative infection, since there were no infections in our series. However, the risk of infection is still present. The prophylactic use of antibiotics provides protection against infection, and the patient's immunological system plays an important role. In this study, no additional antibiotics other than those routinely administered for prophylaxis were prescribed for the patients with a positive culture. In the study by Díaz-de-Rada et al.²⁰, oral antibiotics were given for two weeks to patients with a positive culture. We believe that doing so is not necessary and might be overtreatment in the absence of clinical manifestations of an infection.

Although nonspecific, the C-reactive protein level and erythrocyte sedimentation rate are valuable screening tests to detect postoperative infection. Many studies⁵⁻⁷ have noted that the combination of a normal C-reactive protein level and normal erythrocyte sedimentation rate reliably predicts the absence of infection after elective orthopaedic surgery. Similar to those studies, our investigation found that the C-reactive protein levels were higher early after surgery and then returned to normal in approximately twenty days. In contrast, the erythrocyte sedimentation rate increased during the first week after surgery and was not normalized by twenty days postoperatively. Our findings are in accordance with those in the study by Margheritini et al.²³, who provided data about C-reactive protein and erythrocyte sedimentation rate changes after uncomplicated anterior cruciate ligament reconstruction. Furthermore, the C-reactive protein level and erythrocyte sedimentation rate were similar in patients with contaminated grafts and those with uncontaminated grafts, but this finding should be expected since no patient had a postoperative infection develop. According to the literature, C-reactive protein levels and the erythrocyte sedimentation rate remain elevated in patients with septic arthritis following anterior cruciate ligament reconstruction^{3,4,8-10,24}. Therefore, we agree with those authors who stated that an abnormally elevated C-reactive protein level should raise suspicion of an infection, although a control group of patients with a postoperative infection was not included in our study.

The main limitation of our study according to the post hoc power analysis is that the selected sample sizes were not sufficiently large to show a difference that potentially could be present. Therefore, our results are indicative and should be interpreted within the context of this limitation. However, the study has the advantages of being prospective with a consecutive series of patients, all of whom had the operation done by the same surgeon and all of whom had complete follow-up.

In conclusion, our findings demonstrated that autograft contamination during preparation for anterior cruciate ligament reconstruction can occur at a relatively high rate, and it is important for surgeons performing anterior cruciate ligament

reconstruction to be aware of this fact. The risk for graft contamination is apparently equal between bone-patellar tendon-bone and hamstring tendon autografts. We believe that, in a patient with a contaminated graft, no further treatment is necessary in the absence of clinical signs of infection. Finally, the C-reactive protein level and erythrocyte sedimentation rate following surgery in patients with an implanted contaminated graft but without infection run the same course as in patients with an uncontaminated graft. However, additional studies with a larger number of patients are needed to validate these conclusions. ■

Michael E. Hantes, MD
7 Tabara Street, 41447 Larissa, Greece. E-mail address:
hantesmi@otenet.gr

Georgios K. Basdekis, MD
Sokratis E. Varitimidis, MD
Dimitrios Giotikas, MD
Efthimia Petinaki, MD
Konstantinos N. Malizos, MD
Departments of Orthopaedics (G.K.B., S.E.V., D.G., and K.N.M.)
and Microbiology (E.P.), Medical School, University Hospital of Larissa,
Mezourlo, PC 41110 Larissa, Greece

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